

**EXAMPLE PROCEDURE FOR FIELD-FILTERING SAMPLES FOR
CRYPTOSPORIDIUM ANALYSIS USING
IDEXX FILTA-MAX™ FILTERS**

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Example Procedure for Field-Filtering Samples for *Cryptosporidium* Analysis Using IDEXX Filta-Max™ Filters

1.0 Required Materials

Check to make sure the following materials are available before collecting your sample:

- ☐ Several pairs of new, powder-free latex gloves (Lab Safety Supply, cat. number 16285XL, or equivalent)
- ☐ Sample collection form
- ☐ Filta-Max™ foam filter module (IDEXX, cat. number, FMC 10603) with housing (IDEXX, cat. number, FMC 10504)
- ☐ Electric peristaltic pump (Masterflex L/S variable-speed drive, Cole Parmer, cat. number LC-07554-90, or equivalent with Masterflex L/S Easy Load II pump head, Cole Parmer, cat. number LC-77200-60, or equivalent) (for use only for collection of samples from an unpressurized source)
- ☐ One length of peristaltic pump tubing (Masterflex C-Flex LS 18 tubing, Cole Parmer, cat. number EW-06424-18, or equivalent)
- ☐ Five lengths of 12.7-mm (0.5-in.) internal-diameter clear laboratory tubing (Tygon formula R-3603 [Cole Parmer, cat. number, 06408-18], or equivalent)
- ☐ Five pairs of clamps fit to tubing
- ☐ One coupling to fit 0.5-in. internal diameter tubing
- ☐ Pressure regulator (Watts Regulator, cat. number, 263A, or equivalent)
- ☐ Pressure gauge (maximum operating pressure 120 psi) (Cole Parmer, cat. number, E-68004-05, or equivalent)
- ☐ Water meter (flow totalizer) (Omega Engineering, cat. number, FTB-4005, or equivalent)
- ☐ Flow rate meter (Blue-White, cat. number, F44375LEA 8, or equivalent)
- ☐ Flow control valve (Plast-o-matic, model number #FC050B-1/2-PV)
- ☐ Sample number label
- ☐ Cooler, approximately 16-quart (Coleman, model number, 6216-703, or equivalent)
- ☐ Two large plastic trash bags
- ☐ One 8-lb bag of ice
- ☐ Gel ice pack (VWR, cat. number, 15715-105, or equivalent)
- ☐ Three 13 × 18 inch ziplock bag (Associated Bag, cat. number, 270-42, or equivalent)
- ☐ Strapping tape
- ☐ Two, self-adhesive plastic airbill sleeves
- ☐ Airbill for shipment

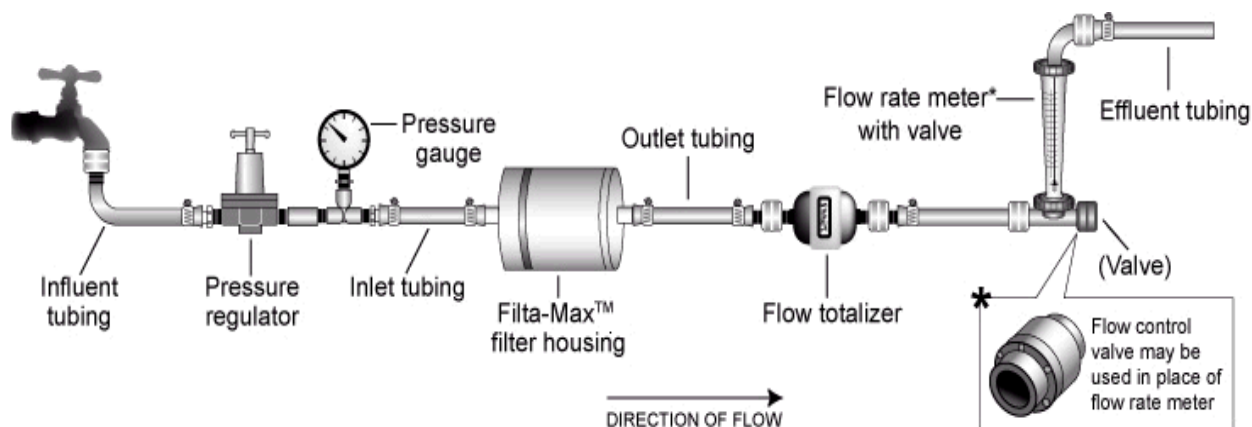
2.0 Collecting the Sample

If the sample will be collected from a pressurized source, use the sample collection procedures in Section 2.1. If the sample will be collected from an unpressurized source, use the sample collection procedures in Section 2.2.

2.1 Sample Filtration from a Pressurized Source

- 2.1.1 Before connecting the sampling system to the tap or source, turn on the tap and allow the water to flow for 2 to 3 minutes or until any debris that has accumulated in the source water lines has cleared or the turbidity in the water becomes visibly uniform. Turn off the tap.
- 2.1.2 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination of the sampling apparatus may bias the final results.
- 2.1.3 Determine the pressure of the water supply using a pressure gauge.
- 2.1.4 Assemble the sampling system, minus the Filta-Max™ filter. For high pressure (> 20 psig) sites, the sampling system should be assembled at the sample port valve in the following order, as shown in **Figure 1** below:
- ☐ Reinforced influent tubing
 - ☐ Pressure regulator
 - ☐ Pressure gauge
 - ☐ Reinforced inlet tubing
 - ☐ Filta-Max™ filter housing
 - ☐ Reinforce outlet tubing
 - ☐ Flow totalizer (mechanical or graduated collection device)
 - ☐ Flow control valve or flow rate meter with valve
 - ☐ Effluent tubing to drain

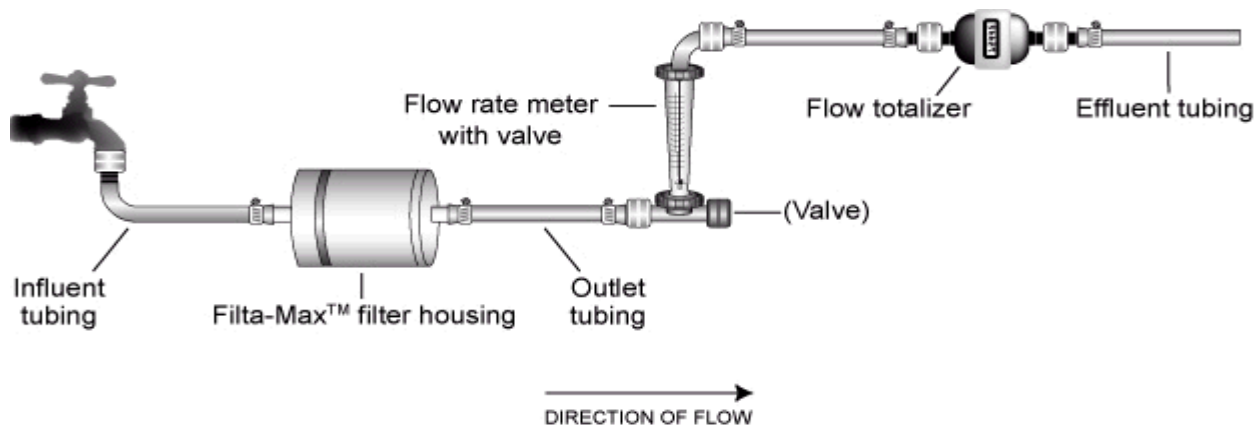
Figure 1. Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (> 20 psig)



For a low-pressure (7.5 to 20 psig) site, the sampling system should be assembled at the sample port valve in the following order, as shown in **Figure 2** below:

- ☐ Influent tubing
- ☐ Filta-Max™ filter housing
- ☐ Outlet tubing
- ☐ Flow rate meter with valve
- ☐ Flow totalizer (mechanical or graduated collection device)
- ☐ Effluent tubing to drain

Figure 2. Sample System Setup for Collecting *Cryptosporidium* Samples from a Pressurized Source (7.5 - 20 psig)











2.1.5 Connect the sampling system, with an empty Filta-Max™ filter housing, to the pressurized water system. Verify that the filter housing is installed so that the end closest to the screw top cap is the inlet and the opposite end is the outlet.

2.1.6 Slowly turn the tap to fully open. Allow a minimum of 20 L to flush the system. During this period, perform the following steps:

2.1.6.1 Measuring flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 3 - 4 L/min (approximately 0.8 - 1 gpm). At high pressure sites, using the pressure regulator, adjust the pressure to a maximum of 120 psi. Observe system for leaks and take the necessary corrective action if any are present. A differential pressure of 7.5 psi is required to create flow through the filter. The recommended differential pressure to produce the flow rate of 3 to 4 L/min is 75 psi. Do not exceed the maximum operating pressure of 120 psig.

2.1.6.2 Record the following information on the sample collection form:


- Public water system (PWS) name
- PWS address
- Sampler name
- Sample ID (optional)

-  Public Water System Identification (PWS ID) number
-  Public Water System facility ID number
-  Facility name
-  Sample collection point ID
-  Sample collection point name
-  Sample collection date
-  Source water type (optional [but required for *E. coli* sample forms])
-  Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both “*Cryptosporidium* field sample” and “*Cryptosporidium* matrix spike” sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.1.6.3 After the system has been flushed, any optional water quality parameters such as temperature, pH, and/or turbidity should be measured now.

2.1.7 Turn off the water at the sample port valve when the flow rate has been adjusted and the system has been flushed.


2.1.8 Record the following information on the sample collection form:

-  Current meter reading

2.1.9 Install the Filta-Max™ filter into the housing and secure the housing cap by hand tightening. Apply gentle pressure to create a seal between the module and the “O” rings in the base and lid of the housing. Excessive tightening is not necessary, and may shorten the life of the “O” rings. A light application of vacuum grease may be used to lubricate the “O” rings, but too much grease will produce a negative effect.




Note! Retain the end caps provided with the filter housing. These caps will be needed to seal the housing for shipment.

2.1.10 Turn on the pressurized water source. Adjust flow to within 3 to 4 L/min, if necessary.

-  Record start time on the sample collection form.




2.1.11 Monitor the water meter. When the targeted volume (typically 10 L [2.64 gal] or 50 L [13.2 gal]) has passed through the Filta-Max™ filter, shut off the water source. Allow the pressure to decrease until the water stops.

2.1.12 Record the following information on the sample collection form:

-  Stop time (when the water was shut off)
-  Final meter reading
-  Comments to laboratory, if needed

2.1.13 Disconnect the inlet end of the filter housing, making sure not to spill any of the water remaining in the capsule. This water is part of your sample.

- 2.1.14 The filter can either be shipped in the filter housing or removed from the filter housing and shipped alone.
- 2.1.15 If the filter will be shipped without the housing, open the housing and dump the filter and any water remaining in the housing into a ziplock bag. Place this bag inside a second ziplock bag and seal.
- 2.1.16 If the filter will be shipped in the housing, seal the inlet and outlet of the housing with the rubber stoppers that were previously saved. Place the filter housing containing the filter in a plastic ziplock bag for shipment.
- 2.1.17 Place a label on the outer ziplock bag containing the filter and using a waterproof pen record the following information:

-  PWS ID
-  Facility name
-  Date of sample collection

Cover the label with clear tape to keep the label dry.

- 2.1.18 Immediately following sample collection, place the bag containing the filter (with or without filter housing) in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill, and replace the ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be $<10^{\circ}\text{C}$ (but not frozen), and the laboratory must have the filter processed within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

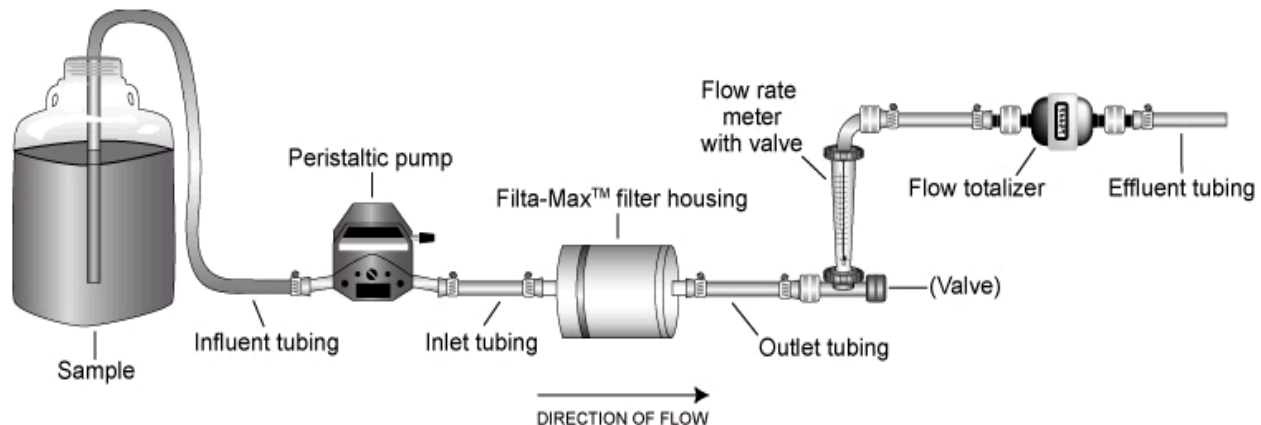
As a recommendation, it is best to have the filter processed by the laboratory within 24 hours of sampling.

2.2 Sample Filtration from an Unpressurized Source



- 2.2.1 Put on a pair of powder-free latex gloves to prevent contamination from outside sources. Any contamination of the sampling apparatus may bias the final results.
- 2.2.2 If sampling from a source of unlimited volume, it may be desirable to pre-flush the sampling system. Assemble the sampling system, minus the Filta-Max™ filter. Verify that the filter housing is installed so that the end closest to the screw top cap is the inlet and the opposite end is the outlet. The sampling system should be assembled in the following order, as shown in **Figure 3** below:
- ☐ Influent tubing
 - ☐ Peristaltic pump
 - ☐ Inlet tubing
 - ☐ Filta-Max™ filter housing
 - ☐ Outlet tubing

- ☐ Flow rate meter with valve
- ☐ Flow totalizer (mechanical or graduated container)
- ☐ Effluent tubing to drain

Figure 3. Sample System Setup for Collecting *Cryptosporidium* Samples from an Unpressurized Source




- 2.2.3 Place the inlet end of the inlet tubing in sample source, away from any walls, bottom, or other environmental surfaces.
- 2.2.4 Turn on the pump and allow a minimum of 20 L to flush the system. If sampling source water from a carboy, continuously refill the carboy as necessary to flush the system. As a recommendation, the carboy should not be removed from the sampling chain and should be refilled using a separate container. Observe the system for leaks and take the necessary corrective action if any are present. During this period, perform the following steps:
 - 2.2.4.1 Measuring flow rate with the flow rate meter or the flow totalizer and a stopwatch, adjust the flow rate to approximately 3 - 4 L/min (approximately 0.8 - 1 gpm) by varying the pump speed or adjusting the valve (if pump is not variable speed).
 - 2.2.4.2 Record the following information on the sample collection form:
 - Public water system (PWS) name
 - PWS address
 - Sampler name
 - Sample ID (optional)
 - Public Water System Identification (PWS ID) number
 - Public Water System facility ID number
 - Facility name
 - Sample collection point ID
 - Sample collection point name
 - Sample collection date

-  Source water type (optional [but required for *E. coli* sample forms])
-  Requested analysis (circle *Cryptosporidium* field sample for routine monitoring sample; circle both “*Cryptosporidium* field sample” and “*Cryptosporidium* matrix spike” sample if you are sending an additional sample with the monitoring sample for matrix spike analysis)

2.2.4.3 Once the system has been flushed, any optional water quality parameters such as temperature, pH, and/or turbidity should be measured now.

2.2.5 Turn off the pump when the flow rate has been adjusted and the system has been flushed. Following flushing, or if flushing is not performed, proceed with the following steps:

2.2.6 Record the following information on the sample collection form:

-  Current meter reading

2.2.7 Install the Filta-Max™ filter into the housing and secure the housing cap by hand tightening. Apply gentle pressure to create a seal between the module and the “O” rings in the base and lid of the housing. Excessive tightening is not necessary, and may shorten the life of the “O” rings. A light application of vacuum grease may be used to lubricate the “O” rings, but too much grease will produce a negative effect.




Note! Retain the end caps provided with the filter housing. These caps will be needed to seal the housing for shipment.

2.2.8 Turn on the peristaltic pump. Record the following on the sample collection form:

-  Start time

2.2.9 Monitor the water meter. When the targeted volume (typically 10 L [2.64 gal] or 50 L [13.2 gal]) has passed through the Filta-Max™ filter or the container is depleted, remove the inlet tubing from the source. Shut off the pump when all the water has been forced through the filter. Allow the pressure to decrease until the water stops. If sampling from a container, add 1 L of reagent water per every 10-L container volume and rinse the sides of the container. Resume pumping until the rinse volume is filtered.




2.2.10 Record the following information on the sample collection form:

-  Stop time (when the pump was shut off)
-  Final meter reading or measured total volume sample, not including reagent rinse volume, if any
-  Comments to laboratory, if needed

2.2.11 Disconnect the inlet end of the filter housing, making sure not to spill any of the water remaining in the capsule. This water is part of your sample.

2.2.12 The filter can either be shipped in the filter housing or removed from the filter housing and shipped alone.

- 2.2.13 If the filter will be shipped without the housing, open the housing and dump the filter and any water remaining in the housing into a ziplock bag. Place this bag inside a second ziplock bag and seal.
- 2.2.14 If the filter will be shipped in the housing, seal the inlet and outlet of the housing with the rubber stoppers that were saved previously. Place the filter housing containing the filter in a plastic ziplock bag for shipment.
- 2.2.15 Place a label on the outer ziplock bag containing the filter and using a waterproof pen record the following information:

-  PWS ID
-  Facility name
-  Date of sample collection

Cover the label with clear tape to keep the label dry.

- 2.2.16 Immediately following sample collection, place the bag containing the filter housing in a refrigerator to chill prior to packing the shipping cooler for shipment. If no refrigerator is available, and the sample will not be shipped for several hours, place the bag in the shipping cooler with ice to chill. Replace the ice before shipping.

Note! Method 1622/1623 requires that the temperature of the sample upon arrival at the laboratory must be $<10^{\circ}\text{C}$ (but not frozen), and the laboratory must have the filter processed within 96 hours of sample collection. If the sample temperature and holding time requirements are not met, then the sample is invalid and must be recollected.

As a recommendation, it is best to have the filter processed by the laboratory within 24 hours of sampling.

3.0 Packing the Sample

- 3.1 Insert two large plastic trash bags into the shipping cooler to create a double liner. Immediately before packing the cooler, create two 4-lb ice packs by putting half of an 8-lb bag of ice into two separate ziplock bags. To prevent leaks place each ice pack into an additional ziplock bag. Gel packs or blue ice may be used instead of wet ice, as long as the sample is maintained in the appropriate temperature range. Seal the ziplock bag, expelling as much air as possible, and secure top with tape.

Note! Shipping companies may delay sample shipments if leakage occurs. Double liners and ziplock bags around ice will prevent leakage and delays.

- 3.2 Place the bag containing the filter housing into the shipping container. Place the ice pack around, but not on, the sample bag to prevent freezing. Inflated, empty sample bags can be placed between the filter housing and the ice packs to prevent the filter from freezing.
- 3.3 If you will be monitoring sample temperature during shipment, place in the cooler the temperature monitoring device (e.g., extra sample bottle for measuring sample temperature upon receipt at the laboratory, thermometer vial, or Thermochron™ iButton). Seal each liner bag by twisting top of bag and tying in a knot.

- 3.4 Peel the backing off one of the plastic airbill sleeves and attach the sleeve to the inside of the cooler lid.



Sign and date the sample collection form

Fold the completed sample collection form, and place it inside the plastic sleeve.

- 3.5 Close the cooler lid, seal the horizontal joints with duct tape, and secure the lid with tape by taping the cooler at each end, perpendicular to the seal.

Note! Shipping companies may delay sample shipments if leakage occurs. Be sure to seal the cooler joints.

- 3.6 Peel the backing off of the second airbill sleeve and attach the sleeve to the outside of the cooler lid. Complete the shipping airbill with the laboratory address, billing information, sample weight, and shipping service. Remove the shipper's copy of the airbill, and place the remainder of the airbill inside the plastic sleeve.

4.0 Shipping and Tracking

- 4.1 Ship samples on the day of collection and use a reliable shipping service for next-day delivery. If samples are not shipped the day of collection, the sample must be maintained at $<10^{\circ}\text{C}$ (but not frozen) by chilling in a refrigerator or cooler filled with ice.
- 4.2 Contact the laboratory to notify them of the sample shipment. Request that the laboratory contact you the next day if the sample is not received.
- 4.3 Using the airbill number on the shipper's copy of the airbill, track the sample shipment using the shipping company's web page or by contacting the shipping company over the phone.
- 4.4 If problems are encountered with the shipment, communicate with the shipping company to resolve, and update the laboratory regarding the status of the shipment.